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Brooke–Spiegler Syndrome Locus Assigned to 16q12-q13

To the Editor:

Brooke–Spiegler syndrome (BSS) is an autosomal dominantly inherited disease, characterized by the development of multiple trichoepitheliomas and cylindromas (Weyers *et al*, 1993).

A major and unique feature of BSS is that of a mixed phenotype with heterogeneity of symptoms and tumor phenotypes in affected families – there have been reports of the coexistence of spiradenomas and trichoepitheliomas (Weyers *et al*, 1993) and familial cylindromas frequently accompanied by trichoepitheliomas (Delfino *et al*, 1991; Burrows *et al*, 1992). Cylindroma and trichoepithelioma have been found in a single nevoid plaque from a patient with BSS (Schirren *et al*, 1995). Mixed differentiation in tumor specimens from two individuals from the same family has also been found (Puig *et al*, 1998). This implies an alteration in the stem cells of the folliculosebaceous-apocrine unit (FSAU) that could be characteristic of this disorder, and where mutations in genes regulating proliferation and differentiation of the putative stem cells would give rise to different combinations of adnexal skin tumors as well as to other neoplasms.

The gene for familial cylindromatosis has been localized to chromosome 16q12-q13 (Biggs *et al*, 1995). Loss of heterozygosity was demonstrated, suggesting that the gene involved is a tumor suppressor. The gene for multiple familial trichoepithelioma has been localized to chromosome 9p21 (Harada *et al*, 1996).

Brooke–Spiegler syndrome demonstrates both cylindromatosis as well as trichoepitheliomas, together with a lot of other phenotypic heterogeneity, including parotid basal cell adenomas, milia, organoid nevi, basal cell carcinomas, and spiradenomas. Whether BSS is a result of mutations in the same genes responsible for familial cylindromatosis and/or trichoepithelioma, or due to the involvement of other, as yet unlocalized, genes would greatly enhance diagnosis and treatment of BSS.

Linkage studies were carried out on lymphocyte DNA from a family comprising six individuals in total, with three affected members. **Figure 1** shows this family with clinical phenotypes in affected individuals. Individual C/BCC exhibits both cylindromas and basal cell carcinomas.

The DNA was amplified using primer pairs from the two candidate regions on chromosomes 9 and 16. Products were electrophoresed on a denaturing urea polyacrylamide gel, and the results analyzed following autoradiography.

DNA was extracted from biopsies taken from tumor samples from one affected individual, amplified with appropriate markers from chromosome 16 and analyzed for loss of heterozygosity.

Eight microsatellite markers were used (between D9S285 and D9S169), spanning the 4 cM candidate interval on chromosome 9, containing the gene for trichoepithelioma, and extending about 2 cM either side. The haplotype obtained excluded linkage of BSS to this region of chromosome 9 in our pedigree.

Thirteen satellite markers (between D16S690 and D16S3253) were used spanning the 6 cM candidate region on chromosome 16 (the location of the gene for cylindromatosis), and extending either side 1–6 cM. **Figure 1** shows the haplotypes segregating through family. The haplotype segregating with syndrome is boxed.

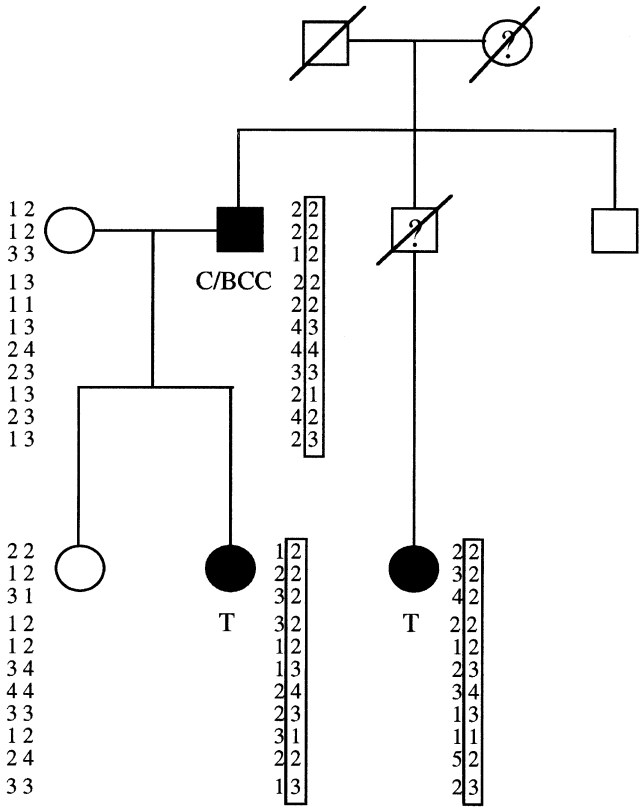

Figure 1. Brooke–Spiegler pedigree indicating clinical phenotypes in affected individuals and haplotypes segregating through family. Haplotype segregating with syndrome is boxed; C, cylindromas; BCC, basal cell carcinomas; T, trichoepitheliomas. Individual C/BCC exhibits both cylindromas and basal cell carcinomas.

Multipoint analysis was carried out using the GENEHUNTER programme (assuming complete penetrance and equal allele frequencies). Results obtained for this small family (LOD score = 1.2) suggest that this region includes a locus involved in the development of BSS.

We suggest therefore that 16q12-q13 includes a locus for BSS and that, given the heterogeneity of the disease, other loci are likely to be involved.

Loss of heterozygosity was demonstrated between markers D16S308 and D16S503 in one tumor sample and between D16S308 and D16S514 in another sample. Together with the linkage results, this provides strong evidence for a susceptibility locus in this family, which is likely to be a tumor suppressor gene.

A database search of this region on 16q identified cDNA at one locus (Cda01g10) where the base sequence is highly similar to a general negative regulator of transcription subunit 1 in Saccharomyces cerevisiae. Other candidate genes within this region were found: the retinoblastoma-related gene, which has been

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mapped to 16q12.2 (Mayol *et al*, 1993) and a growth inhibitory factor in the human brain, metallothionein 3 (Tsuji *et al*, 1992).

Other syndromes, with features in common with BSS (e.g., Cowden's, Gorlin's, and Muir–Torre syndromes) and whose genes have been localized would be the most obvious candidate regions to begin screening for contributing loci. Muir–Torre syndrome, characterized by sebaceous gland tumors and a minimum of one visceral malignant tumor, has been shown to involve several loci on 2p,3p,5q,9p,17p,18q (Peris *et al*, 1997) with demonstration of loss of heterozygosity and microsatellite instability.

Once such loci have been excluded a genome wide screen and subsequent linkage analysis would identify other loci involved in the development of BSS. Mutational and LOH analysis of these genes will help to determine mechanisms and paths of cell development involved in BSS.

In summary, our mapping of a locus for BSS to chromosome 16q12-q13 is the first step in the identification of the gene(s) involved; preliminary evidence indicates that the candidate gene described here is a tumor suppressor.

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REFERENCES

Biggs PJ, Wooster R, Ford D, *et al*: Familial cylindromatosis (turban tumour syndrome) gene localised to chromosome 16q12-q13: evidence for its role as a tumour suppressor gene. *Nature Genetics* 11:441–443, 1995

Burrows NP, Jones RR, Smith NP: The clinicopathological features of familial cylindromas and trichepitheliomas (Brooke-Spiegler syndrome): a report of two families. *Clin Exp Dermatol* 17:332–336, 1992

Delfino M, D'Anna F, Ianiello S, Donofrio V: Multiple hereditary trichepithelioma and cylindroma (Brooke-Spiegler syndrome). *Dermatologica* 183:150–153, 1991

Harada H, Hashimoto K, Ko MSH: The gene for multiple familial trichepithelioma maps to chromosome 9p21. *J Invest Dermatol* 107:41–43, 1996

Mayol X, Neal GE, Davies R, Romero A, Domingo J: Cloning of a new member of the retinoblastoma gene family (pRb2) which binds to the E1A transforming domain. *Oncogene* 8:2561–2566, 1993

Peris K, Onorati MT, Keller G, *et al*: Widespread microsatellite instability in sebaceous tumours of patients with the Muir-Torre syndrome. *Br J Dermatol* 137:356–360, 1997

Puig L, Nadal C, Fernandez-Figueras MT, Alegre M, de Moragas JM: Brooke-Spiegler syndrome variant: segregation of tumour types with mixed differentiation in two generations. *Am J Dermatopathol* 20:56–60, 1998

Schirren CG, Worle B, Kind P, Plewig GJ: A nevoid plaque with histological changes of trichoepithelioma and cylindroma in Brooke-Spiegler syndrome. An immunohistochemical study with cytokeratins. *Cutan Pathol* 22:563–569, 1995

Tsuji S, Kobayashi H, Uchida Y, Ihara Y, Miyatake T: Molecular cloning of human growth inhibitory factor cDNA and its down-regulation in Alzheimer's disease. *EMBO J* 11:4843–4850, 1992

Weyers W, Nilles M, Eckert F, Schill WB: Spiradenomas in Brooke-Spiegler syndrome. *Am J Dermatopathol* 15:156–161, 1993

Desmosome Assembly and Keratin Network Formation After Ca^{2+}/Serum Induction and UVB Radiation in Hailey–Hailey Keratinocytes

To the Editor:

Hailey–Hailey disease (HH) is an autosomal dominant inherited blistering disorder of the skin. Ultrastructural and histologic data have suggested that the cause for HH may lie in a defect of one of the protein components or the process of desmosome assembling (Gottlieb and Lutzner, 1970; Thies *et al*, 1972). This got support from immunofluorescence studies with HH epidermis which showed a diffuse cytoplasmatic staining of desmoplakin, desmoglein, and plakoglobin in acantholytic cells, whereas vinculin (adherens junctions-associated) and connexin (gap junctions) were normally distributed at the cell surface (Burge and Garrod, 1991; Setoyama *et al*, 1991a; Harada *et al*, 1994; Hashimoto *et al*, 1995). In not yet detached preacantholytic cells, which occur sporadically in unaffected epidermis of HH patients, desmosomal proteins are already diffusely spread throughout the cytoplasm while their adherens junctions and actin filament network appear normal (Setoyama *et al*, 1991b; Metze *et al*, 1996). Blistering in clinically unaffected skin can be provoked by cellular stress such as suction and UVB radiation (De Dobbeleer and Achten, 1979; Linse and Richard, 1990a, b; Burge *et al*, 1991).

It is controversially discussed whether the first event in HH disease is the lost of keratin filament binding to the desmosomal plaque or the splitting of the desmosomes into two halves (Thies *et al*, 1972; De Dobbeleer and Achten, 1979; Metze *et al*, 1996); however, although desmosomal components seem to be involved in reduced cell–cell adhesion of HH keratinocytes, linkage analysis of HH to 3q21–24 (Ikeda *et al*, 1994; Richard *et al*, 1995) helped to exclude a number of potential candidate genes such as the two clusters of keratin genes and the constitutive desmosomal proteins desmoplakin (6p21), desmocollin and desmoglein (18q21.1), plakoglobin (17q21–22) and the accessory plaque-proteins plakophilin 1 (1q31), plakophilin 2 (12p13) and plakophilin 3 (11p15). Nevertheless, these data are suggestive that desmosomal proteins are at least indirectly affected in HH disease.

In monolayer culture, desmosome assembly and keratin network reorganization can be studied by performing a calcium shift from low to high calcium medium (0.1 mM to 1.5–1.8 mM). Under low calcium conditions, desmosomal proteins are diffusely distributed in the cytoplasm whereas keratin filaments are found in bundles perinuclearly. Within minutes after the shift, desmosomal components appear at the cell–cell borders and take part in desmosome formation. Simultaneously the keratin filaments reorganize by attaching to the desmosomes and spreading throughout the cytoplasm (Watt *et al*, 1984; O'Keefe *et al*, 1987). We used this cell culture model to study in HH keratinocytes after Ca^{2+}/serum shift the formation of desmosomes and the biochemical properties of prominent desmosomal proteins. Additionally we stressed HH keratinocytes by UVB radiation and observed desmosome formation and reorganization of keratin network thereafter.

After informed consent biopsies were taken from the axilla or groin including lesional as well as neighboring unaffected skin of three HH patients belonging to two unrelated German families. Control biopsies were obtained from normal adults during routine surgical procedures. Keratinocytes were isolated according to standard procedures and taken up in serum free medium with 0.09 mM Ca^{2+} (SFM, Life Technologies, Eggenstein, Germany), supplemented with 5 μg gentamicin per ml, 100 U penicillin per ml, and 100 μg streptomycin per ml and grown at 37°C, 5% CO_2 and saturated atmospheric humidity. Desmosome formation was induced by replacing SFM with Dulbecco's modified eagle medium that was complemented with 10% fetal calf serum

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